

Remarks

Please reconsider the application in view of the following remarks. Applicant thanks the Examiner for carefully considering this application.

Disposition of Claims

Claims 1-20 were pending in this application. Claims 1-13 have been canceled. Therefore, claims 14-20 are pending after the amendments. Claim 14 is independent. The remaining claims depend, directly or indirectly, from claim 14.

Rejection(s) under 35 U.S.C. § 103

Claims 1-20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Koketsu, et al. (J. Carbohydrate Chemistry, 1995, Vol. 14, No., p.833-841) (hereinafter "Koketsu 1995") and SCORE search result, in view of Yamamoto et al. (JP 08099988 A, 1996, abstract) (hereinafter "Yamamoto 1996") and further in view of Inazu, et al. (Peptide Science 1998, M. Kondo Edition, p.153-156) (hereinafter "Inazu") and Koketsu, et al. (The Journal of Food Science, 1993, Vol. 58, No. 4, pp. 743-747) (hereinafter "Koketsu 1993") and Yamamoto, K. (Journal of Bioscience and Bioengineering, 2001, Vol. 92, No. 6, pp. 493-501) (hereinafter "Yamamoto 2001"). Claims 1-13 have been canceled, rendering this rejection moot with respect to these claims. With respect to claims 14-20, this rejection is respectfully traversed.

The present invention relates to methods for preparing asparagine-linked oligosaccharide derivatives by subjecting delipidated egg yolk to a two-step enzymatic process, i.e., first with a protease and then with a peptidase. The methods include an isolation step between the protease reaction and the peptidase reaction.

In order to produce a single amino acid (asparagine)-linked oligosaccharide derivatives, methods of the invention use two different enzymes – first with a protease (proteinase) that is more active towards proteins, and then with a peptidase that is more active towards peptide fragments. The use of two different enzymes, each specific for different sizes of substrates, makes the production more efficient than the conventional one-enzyme approach.

More importantly, according to methods of the invention, the products from the protease reaction are isolated before the second step of peptidase reaction. The isolation step significantly improves the yields of the asparagine-linked oligosaccharide derivatives.

As shown in the Example 1 and the comparative Example in the Declaration under 37 C.F.R. 1.132 filed herewith, a method with an isolation step between the two enzyme digestion steps produced 75% more products (13.3 mg), as compared with a method without an isolation step (7.63 mg). More importantly, the better yield with a method of the invention is achieved with less enzyme (43 mg + 21.5 mg of actinase in Example 1 versus 144 mg + 72 mg of actinase in the comparative example). It is clear that when the isolation step is omitted, the actinase is not as efficient. As a result, the yield is substantially lower. Thus, with an isolation step, a method of the invention produces unexpectedly superior results, as compared with a method without an isolation step.

Specifically, claim 14 requires, *inter alia*, “(a) treating a delipidated egg yolk with a protease to obtain a mixture of peptide-linked oligosaccharides; (b) isolating the mixture of peptide-linked oligosaccharides; (c) treating the isolated mixture of peptide-linked oligosaccharides with a peptidase to obtain a mixture of asparagine-linked oligosaccharides.”

Koketsu 1995 teaches a one-step enzymatic process for preparing sialylglycopeptides using Orientase to treat the delipidated egg yolk. (*See* page 838, under “Experimental”). Thus, Koketsu 1995 does not use two different enzymes selected for different sizes of the substrates. More importantly, Koketsu 1995 does not teach a method that includes an isolation step.

Yamamoto 1996 teaches a method for preparing sialic acid-containing oligosaccharides, not asparagine-linked oligosaccharides. Specifically, Yamamoto 1996 teaches that conventional methods for preparing oligosaccharides by directly treating delipidated egg yolk with proteases require a large quantity of proteases. Therefore, Yamamoto 1996 teaches a method to solve this problem by first extracting the delipidated egg yolk with water or a salt solution before enzyme treatments. (*See* paragraphs [0004], [0005], and [0007]). However, Yamamoto 1996 does not teach a two-step method, including an isolation step, for preparing asparagine-linked oligosaccharide derivatives as recited in claim 14.

Therefore, a combination of Koketsu 1995 with Yamamoto 1996 would not teach or suggest every limitation of claim 14. Inazu does not teach or suggest that which is missing in Koketsu 1995 and Yamamoto 1996. The Examiner relies on Inazu to teach introducing a lipophilic protective group and subjecting the mixture of asparagine-linked oligosaccharide derivatives to a fractionating chromatography.

Koketsu 1993 also does not teach or suggest that which is missing in Koketsu 1995, Yamamoto 1996, and Inazu, as evidenced by the fact that the Examiner relies on Koketsu 1993 to teach treating with ethanol, separating using reverse-phase column, hydrolyzing, and adding sialyloligosaccharides to drugs and foods.

Similarly, Yamamoto 2001 also does not teach or suggest that which is missing in all the above references, as evidenced by the fact that the Examiner relies on Yamamoto 2001 to teach the motivation for introducing a lipophilic protection group and hydrolyzing the asparagine-linked oligosaccharides and the reasonable expectation of success of treating delipidated egg yolk with actinase E.

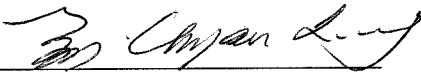
Thus, Koketsu 1995 and SCORE search result in view of Yamamoto 1996, further in view of Inazu, Koketsu 1993, and Yamamoto 2001 would not teach or suggest every limitation of claim 14. Therefore, claim 14 is patentable over Koketsu 1995 and SCORE search result in view of Yamamoto 1996, further in view of Inazu, Koketsu 1993, and Yamamoto 2001. The dependent claims should also be patentable for at least the same reasons. Accordingly, withdrawal of this rejection is respectfully requested.

Conclusion

Applicant believes this reply is fully responsive to all outstanding issues and places this application in condition for allowance. If this belief is incorrect, or other issues arise, the Examiner is encouraged to contact the undersigned or his associates at the telephone number listed below. Please apply any charges not covered, or any credits, to Deposit Account 50-0591 (Reference Number 17563/004001).

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Respectfully submitted,

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